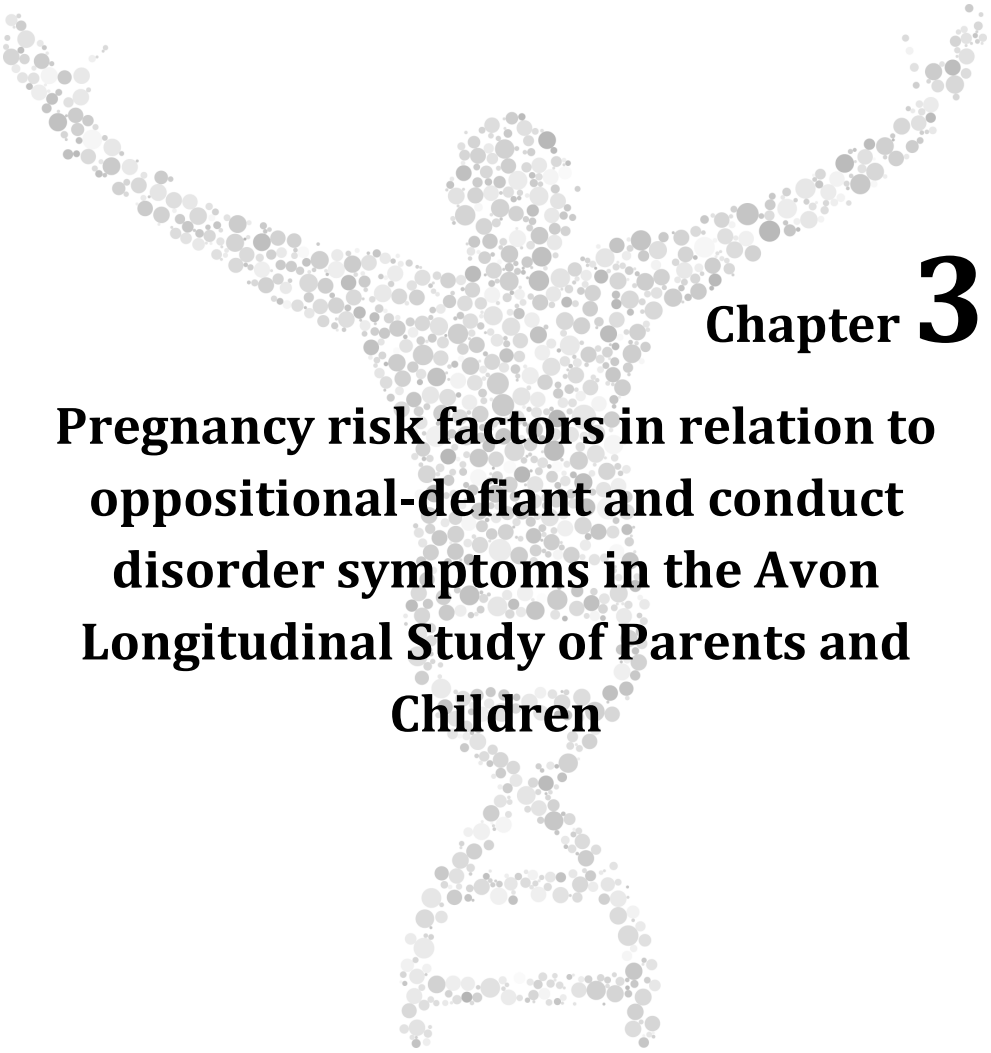
Gene-environment interactions in disruptive behaviors

Ruisch, Hyun

DOI:
10.33612/diss.136546089



Chapter 3

Pregnancy risk factors in relation to oppositional-defiant and conduct disorder symptoms in the Avon Longitudinal Study of Parents and Children

Published as:
Ruisch IH, Buitelaar JK, Glennon JC, Hoekstra PJ, Dietrich A. Pregnancy risk factors in relation to oppositional-defiant and conduct disorder symptoms in the Avon Longitudinal Study of Parents and Children. Journal of Psychiatric Research. 2018; 101: 63-71.

Acknowledgements, funding & declarations

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and this research is supported by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 603016 (MATRICS). I. Hyun Ruisch, Pieter J. Hoekstra, and Andrea Dietrich reported no financial interests or potential conflicts of interest. Jan K. Buitelaar was a consultant to/member of advisory board of/and/or speaker for Janssen-Cilag BV, Eli Lilly, Shire, Novartis, Roche and Servier. Jeffrey C. Glennon has in the past three years been a consultant to Boehringer Ingelheim GmbH. Neither Jan K. Buitelaar nor Jeffrey C. Glennon are employees of any of these companies, and neither are stock shareholders of any of these companies. I. Hyun Ruisch wrote a manuscript draft and conducted the statistical analyses. Pieter J. Hoekstra, Andrea Dietrich, and I. Hyun Ruisch designed the study. Pieter J. Hoekstra, Andrea Dietrich, Jeffrey C. Glennon and Jan K. Buitelaar provided general feedback as well as emphasized specific issues and potential confounds in the discussion section of the article manuscript. All authors have contributed to and approved the final manuscript.

Abstract

Background: Pregnancy factors have been implicated in offspring oppositional-defiant disorder (ODD) and conduct disorder (CD) symptoms. Literature still holds notable limitations, such as studying only a restricted set of pregnancy factors, use of screening questionnaires which assess broadly defined outcome measures, and lack of control for disruptive behavior comorbidity and genetic confounds. We aimed to address these gaps by prospectively studying a broad range of pregnancy factors in relation to both offspring ODD and CD symptomatology in the Avon Longitudinal Study of Parent and Children. **Methods:** Outcomes were ODD and CD symptom scores at age 7;9 years using the Development and Well-Being Assessment interview. We analyzed maternal (N≈6,300) and teacher ratings (N≈4,400) of ODD and CD scores separately using negative binomial regression in multivariable models. Control variables included comorbid attention-deficit/hyperactivity disorder symptoms, ODD or CD symptoms as appropriate, and genetic risk scores based on an independent CD genome-wide association study. **Results:** Higher ODD symptom scores were linked to paracetamol use (IRR=1.24 [98.3% confidence interval 1.05-1.47], P=0.002, teacher ratings) and life events stress (IRR=1.22 [1.07-1.39], P=0.002, maternal ratings) during pregnancy. Higher CD symptom scores were linked to maternal smoking (IRR=1.33 [1.18-1.51], P<0.001, maternal ratings), life events stress (IRR=1.24 [1.11-1.38], P<0.001, maternal ratings) and depressive symptoms (IRR=1.14 [1.01-1.30], P=0.006, maternal ratings) during pregnancy. **Conclusions:** Common and potentially preventable pregnancy risk factors were independently related to both offspring ODD and CD symptomatology in children from the general population. Future studies should further address genetic confounds and confounding by environmental factors later in life.

Introduction

Oppositional-defiant disorder (ODD) and conduct disorder (CD) are disruptive behavior disorders with an estimated pediatric prevalence of about 2 to 16% (1–3). Symptoms as described in the Diagnostic and Statistical Manual of Mental Disorders (DSM) include angriness, defiant behavior, and vindictiveness in the case of ODD, and aggression, destruction, deceitfulness, and severe rule violations in the case of CD (3). Not surprisingly, such behaviors may cause considerable burden to affected families and society at large.

Both genetic (4,5) and environmental (6) risk factors are involved in ODD and CD. Because ODD and CD can be viewed as the extreme outcomes along continuous traits distributed within the general population it is important to investigate these traits in population samples. Adverse environmental exposures can be considered at all stages of life, even before birth. In this regard, specific pregnancy factors such as maternal smoking (7–9), alcohol consumption (10,11), paracetamol use (12,13), and maternal internalizing problems (14,15) have been linked to ODD and CD symptoms by a number of studies in the general population, including the Avon Longitudinal Study of Parents and Children (ALSPAC). In addition, low birth weight and preterm birth (16) have been linked to related disruptive behavior while common infections (17,18) or antidepressant use (19) during pregnancy constitute adversities related to more general neurodevelopment. However, notable limitations still exist in the current literature. First, multiple pregnancy factors might be related to each other. For example anxiety and smoking during pregnancy have been found to be related (20), the use of multiple intoxicating substances occurs frequently (21), and medication use is obviously linked to its prescription indications. Second, high rates of comorbidity among disruptive behavioral disorders (1) poses another problems by implying that reported associations with ODD or CD could very well be driven by comorbid attention-deficit/hyperactivity disorder (ADHD), which occurs in up to 50% of ODD / CD cases. Moreover, as the nature of CD is more severe than ODD, it is also useful to investigate risk factors for ODD or CD independent of each other. Furthermore, maternal behaviors and lifestyle during pregnancy may be related to the same genetic factors that also predispose offspring to disruptive behavior (22,23). Yet, currently, only few studies (7,12,22) have applied some form of control for genetic confounds. Finally, the majority of studies addressing pregnancy factors for ODD and CD symptoms in the general population used screening questionnaires (24) rather than more accurate diagnostic assessments. This also makes it more difficult to distinguish risk factors for ODD or CD specifically as above mentioned.

With the present study we aimed to address these limitations in the current literature and used the well-powered, prospective ALSPAC general population cohort to study a broad range of previously implicated pregnancy factors concurrently in relation to offspring ODD and CD traits.

Methods

The ALSPAC sample

ALSPAC is an ongoing, prospective, longitudinal birth cohort, which initially recruited 14,541 pregnant women in Avon, UK with expected delivery dates from April 1991 to December 1992 and their subsequently born children. At the time of recruitment mothers were between age 16 and 45 and represented about 85% of pregnant women in the catchment area. When children reached the age of 7, the initial sample was enriched with eligible cases who had failed to join the study initially. This resulted in an enrollment of an additional 713 children. Longitudinally collected data comprised a wide range of phenotypic and environmental measures, as well as biological samples and (epi)genetic data. Further details regarding recruitment, study design, and generalizability have been reported elsewhere (25–27). Ethical approval for the ALSPAC-study was obtained from the ALSPAC Ethics and Law Committee as well as the Local Research Ethics Committees. Details on the ethics committee's and institutional review boards that approved aspects of the study can be found at http://www.bristol.ac.uk/alspac/researchers/research-ethics/. For the present study we included subjects with available data for childhood disruptive behavior and a number of pregnancy complications. We excluded 136 subjects with a total IQ score < 70.

Measures

Pregnancy factors. Data was collected prospectively through maternal questionnaires at 18 weeks gestation and by using information from medical records during labor. We selected those pregnancy related risk factors from the ALSPAC database that had been linked in the literature to disruptive behavior and/or more general neurodevelopment: frequently used substances (maternal smoking (7–9), alcohol consumption (10,11), use of cannabis or hard drugs (28,29)), frequently used medication (the commonly used analgesics paracetamol (12,13) and aspirin (30,31); medication for infection (32), anxiety (33,34), or depression (19,34)), occurrence of common infections (influenza (17), candidiasis (18), and urinary tract infections (35)), presence of anxiety and mood problems during pregnancy (stress resulting from life events (15,36), anxiety (15,37), and depression symptoms (14)), and low birth weight (16) and preterm birth (16). **Supplementary Table S1** describes the variables used in our study. In addition, please note that the ALSPAC study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/).

Attention-deficit and disruptive behavior disorders (ODD, CD, ADHD) symptoms were assessed by the Development and Well-Being Assessment (DAWBA) (38). The DAWBA is a psychiatric-diagnostic interview with the parents, complemented by a questionnaire completed by the child's teacher, assessing psychopathology in children and adolescents with good validity (38). Individual symptoms, derived from the Diagnostic and Statistical Manual of Mental Disorders (DSM) version IV (39), were rated on a three point scale (0-2). Symptom scores for ODD (range 0-18), CD (possible range 0-14 for maternal ratings and 0-24 for teacher ratings), and ADHD (range 0-36 for maternal ratings and 0-38 for teacher ratings) were assessed at the age of 7 years and 9 months.

Genetic risk scores. Details regarding genotyping quality control procedures are described in the **supplementary material**. In summary, genotyping was carried out using the Illumina HumanHap550 beadchip array and subsequent imputation with Impute2 v2.2.2 software, and our dataset included genotype data for 8,941 children. Full methods for generating genetic risk scores are described in the **supplementary material**. In short, we calculated genetic risk scores based on the top results of an independent genome-wide association study (GWAS) on CD (40). We included 17 SNPs that were not in linkage disequilibrium (r^2 < 0.6) and reached a P-value < 1.00E-05 for either a CD symptom count or a CD case status in the original GWAS. Genetic risk scores were weighted by explained variance, which takes into account SNP effect size as well as allele frequency (41).

Analyses

We analyzed ODD and CD symptom scores as well as maternal and teacher ratings separately resulting in a total of four outcomes for our analyses. To control the family-wise error rate for multiple comparisons, we adjusted the significance threshold for the number of effective tests by using the eigenvalues of the correlation matrix of our four outcomes (42). The eigenvalues were computed using an online script available at http://gump.qimr.edu.au/general/daleN/matSpD/. The corrected alpha level obtained by this procedure was 0.017. Given the positively skewed and overdispersed outcome data, we used negative binomial regression (43,44). Negative binomial regression uses a logarithmic link function and when regression coefficients are exponentiated an incidence rate ratio (IRR) is obtained. Predictors were dichotomized by creating a contrast between any versus no exposure for categorical variables, and creating a median-split for scores of life events stress, anxiety, and depression. As we used dichotomized predictors the IRR gives the ratio of ODD or CD symptom scores between exposed and unexposed subjects. For example an IRR of 1.50 indicates that exposed subjects are predicted to have a 50% higher symptom score than unexposed subjects. Statistical analyses were carried out with STATA v14.1 (45). Individual pregnancy factors were first screened for relevance regarding ODD and CD. When an individual pregnancy factor reached a P-value < 0.1 it was carried forward into analyses concerning multiple pregnancy factors. Thus, multiple pregnancy factors (all relevant individual factors) were then adjusted for each other by modelling them simultaneously. As control variables we included offspring sex, socioeconomic status (low socioeconomic status was defined as the lowest two social classes based on occupation (46)), young maternal age (age < 20 at delivery), and single parent status during pregnancy (see **Table S1**) in all analyses because these were associated with offspring ODD and/or CD symptom scores in our sample. We also included offspring ADHD symptom scores in all analyses, to identify pregnancy factors for ODD and CD independent from comorbid ADHD. As a next step we additionally adjusted ODD and CD symptom scores for each other, to further investigate which pregnancy factors load specifically on ODD or CD. In the last set of analyses we addressed potential genetic confounding by adding genetic risk scores to the models. Note that approximately 70% of the study sample had been genotyped, reducing the sample sizes for this last set of analyses. Finally, we conducted sensitivity analyses to further investigate potential confounding factors for paracetamol use and smoking during

pregnancy. In the case of paracetamol use we additionally adjusted our models for common maternal infections (influenza, candidiasis, urinary tract infections) and related medication use (aspirin use, medication for infection) and regarding smoking during pregnancy we additionally controlled for low birth weight, as the relationship between these two factors has been well known (47,48).

Results

Summary statistics

Table 1 lists summary statistics for our study sample. From the originally enrolled 14,541 pregnancies, maternal and teacher rated ODD and CD symptom scores were available for approximately 8,000 and 6,300 subjects respectively. Cross-tabulated with the included pregnancy factors and control variables in our multivariable analyses this resulted in maximum sample sizes of 6,321 (ODD) and 6,306 (CD) for maternal rated, and 4,431 (ODD) and 4,422 (CD) for teacher rated symptom scores. Subjects excluded because of missing pregnancy or control variable data showed on average higher ODD and CD symptom scores than included subjects with complete data (**Supplementary Table S2** lists differences between included and excluded subjects).

	Maternal rated symptom scores ***(N=6,270 max.)***	**Teacher rated symptom scores** ***(N=4,422 max.)***
Offspring age at outcome assessment	7 years, 9 months	7 years, 9 months
Offspring male sex	3,245 (51.8%)	2,248 (50.8%)
Maternal age < 20 at delivery	100 (1.6%)	111 (2.5%)
Maternal low socioeconomic status	1069 (17.0%)	872 (19.7%)
Maternal single parent status	310 (4.9%)	252 (5.7%)
Offspring genetic risk score for CD	0.02 ± 0.09 (-0.27 to 0.35)	0.02 ± 0.09 (-0.26 to 0.35)
Offspring ODD symptom score (DAWBA, 7;9 years)	1.35 ± 2.80 (0 to 18)	1.39 ± 3.07 (0 to 18)
Offspring CD symptom score (DAWBA, 7;9 years)	0.55 ± 1.03 (0 to 10)	0.34 ± 1.24 (0 to 16)
Offspring ADHD symptom score (DAWBA, 7;9 years)	4.80 ± 6.69 (0 to 36)	6.15 ± 7.90 (0 to 38)
Pregnancy factors		
Maternal smoking	1,168 (18.6%)	979 (22.1%)
Alcohol use	3,504 (55.9%)	2,450 (55.4%)
Cannabis or hard drugs use	132 (2.1%)	88 (2.0%)
Influenza	872 (13.9%)	593 (13.4%)
Candidiasis	803 (12.8%)	633 (13.7%)
Urinary tract infection	394 (6.3%)	292 (6.6%)
Medication for infection	805 (12.9%)	586 (13.3%)
Paracetamol use	3,321 (53.0%)	2,389 (54.0%)
Aspirin use	285 (4.5%)	201 (4.5%)
Life event stress score	8.12 ± 7.28 (0 to 61)	8.20 ± 7.35 (0 to 61)
Anxiety symptom score (CCEI)	4.62 ± 3.36 (0 to 16)	4.76 ± 3.46 (0 to 16)
Depression symptom score (EPDS)	6.37 ± 4.52 (0 to 28)	6.64 ± 4.68 (0 to 28)
Medication for anxiety or depression	54 (0.9%)	49 (1.1%)
Low birth weight	278 (4.5%)	204 (4.7%)
Preterm birth	317 (5.1%)	239 (5.4%)

Table 1: Summary statistics of our study sample. CD: conduct disorder. ODD: Oppositional defiant disorder. ADHD: attention-deficit/hyperactivity disorder. DAWBA: Development and Well-Being Assessment of childhood psychopathology (38). CCEI: Crown-Crisp Experience Index subscale of anxiety (49). EPDS: Edinburgh Postnatal Depression Scale of depression symptoms, which has also been validated for prenatal use (50,51). Pregnancy factors were prospectively measured at 18 weeks gestation, whereas birth weight and length of gestation were measured perinatally. N (% valid) or mean ± SD (range). For further variable definitions see **Supplementary Table S1**.

Univariable regression analyses

Identification of relevant individual pregnancy factors. See **Table 2** for all results. The following pregnancy factors reached the threshold (P<0.1) for inclusion in subsequent multivariable models: maternal smoking, alcohol use, cannabis or hard drug use, paracetamol use, life events stress scores, anxiety scores and depression scores.

	ODD symptom scores				CD symptom scores			
	Maternal rated (N=6,772 max.)		Teacher rated (N=4,794 max.)		Maternal rated (N=6,775 max.)		Teacher rated (N=4,794 max.)	
	IRR (95% CI)	P	IRR (95% CI)	P	IRR (95% CI)	P	IRR (95% CI)	P
Substance use during pregnancy								
Maternal smoking	1.16 (1.03-1.31)	**0.015**	1.20 (1.04-1.39)	**0.014**	1.43 (1.29-1.57)	**<0.001**	1.21 (0.97-1.51)	**0.091**
Alcohol use	1.08 (0.97-1.20)	0.147	1.03 (0.90-1.17)	0.708	1.09 (1.00-1.19)	**0.040**	1.17 (0.95-1.44)	0.150
Cannabis or hard drug use	1.49 (1.09-2.03)	**0.012**	1.31 (0.94-1.80)	0.115	1.34 (1.04-1.74)	**0.025**	1.46 (0.80-2.69)	0.221
Maternal infections and related medication use during pregnancy								
Influenza	0.98 (0.85-1.14)	0.818	0.93 (0.77-1.14)	0.506	1.00 (0.89-1.13)	0.996	0.91 (0.67-1.23)	0.537
Candidiasis	1.11 (0.96-1.28)	0.152	1.02 (0.86-1.19)	0.851	1.10 (0.98-1.25)	0.109	0.98 (0.74-1.32)	0.913
Urinary tract infection	1.04 (0.85-1.28)	0.707	1.15 (0.92-1.43)	0.221	1.06 (0.89-1.27)	0.488	1.22 (0.85-1.76)	0.280
Medication for infection	0.92 (0.80-1.07)	0.281	1.05 (0.90-1.24)	0.526	1.08 (0.96-1.21)	0.217	1.25 (0.96-1.62)	0.102
Paracetamol use	1.04 (0.94-1.16)	0.402	1.21 (1.06-1.38)	**0.004**	1.14 (1.05-1.24)	**0.002**	1.25 (1.01-1.53)	**0.038**
Aspirin use	1.05 (0.84-1.31)	0.687	0.99 (0.72-1.37)	0.954	1.02 (0.85-1.23)	0.818	1.03 (0.66-1.61)	0.908
*Maternal mental health **problems** during pregnancy*								
Life events stress score	1.29 (1.16-1.42)	**<0.001**	1.08 (0.95-1.22)	0.268	1.32 (1.21-1.43)	**<0.001**	1.18 (0.96-1.46)	0.118
Anxiety symptom score	1.22 (1.10-1.36)	**<0.001**	1.10 (0.97-1.26)	0.149	1.22 (1.12-1.33)	**<0.001**	1.05 (0.85-1.30)	0.652
Depression symptom score	1.19 (1.07-1.32)	**0.001**	1.12 (0.99-1.28)	**0.078**	1.26 (1.16-1.37)	**<0.001**	1.17 (0.95-1.45)	0.146
Medication for anxiety or depression	1.22 (0.79-1.90)	0.374	1.07 (0.60-1.91)	0.818	1.20 (0.79-1.82)	0.387	1.24 (0.32-4.85)	0.760
Other								
Low birth weight	1.02 (0.81-1.29)	0.868	0.93 (0.70-1.24)	0.622	0.86 (0.70-1.06)	0.157	0.97 (0.57-1.65)	0.919
Preterm birth	0.86 (0.70-1.05)	0.131	0.98 (0.76-1.27)	0.883	0.87 (0.72-1.04)	0.133	0.90 (0.59-1.39)	0.650

Table 2: Pregnancy factors in relation to offspring ODD and CD symptom scores. All individual pregnancy and birth complications were analyzed separately and all analyses were adjusted for offspring sex, socioeconomic status, young maternal age at delivery, single parent status and comorbid attention-deficit/hyperactivity disorder symptom scores. CD: conduct disorder. ODD: Oppositional defiant disorder. IRR: incidence rate ratio. Bold *P*-values (P<0.10) indicate selected variables carried forward to the multivariable analyses of multiple pregnancy factors (simultaneously).

Multivariable regression analyses

Multiple pregnancy factors adjusted for each other. See **Table 3** for all results**.** When adjusted for all other included pregnancy factors in the multivariable models, maternal paracetamol use (P=0.002 for teacher ratings) and life events stress scores during pregnancy (P<0.001 for maternal ratings) were linked to higher ODD symptom scores whereas maternal smoking (P<0.001 for maternal ratings), life events stress scores (P<0.001 for maternal ratings) and depressive symptom scores during pregnancy (P=0.011 for maternal ratings) were linked to higher CD symptom scores.

Additional adjustment for respective comorbid ODD or CD symptom scores. See **Table 4** for all results. In addition to controlling for comorbid ADHD symptom scores, we adjusted for either comorbid ODD or CD symptom scores and all findings from the previous models remained significant. That is, paracetamol use (P=0.002 for teacher ratings) and life events stress scores (P=0.002 for maternal ratings) were significant predictors of higher ODD symptom scores (independent of comorbid CD symptom scores) whereas maternal smoking (P<0.001 for maternal ratings), life events stress scores (P<0.001 for maternal ratings) and depression symptom scores (P=0.006 for teacher ratings) were significant predictors of higher CD symptom scores (independent of comorbid ODD symptom scores).

Additional adjustment for genetic risk scores. See **Table 5** for all results. After controlling for comorbid ADHD, and respective ODD or CD symptom scores, as well as genetic risk scores, findings from previous models remained significant. Paracetamol use (P=0.015 for teacher ratings) and life events stress scores (P=0.004 for maternal ratings) were significant predictors of higher ODD symptom scores whereas maternal smoking (P<0.001 for maternal ratings), life events stress scores (P<0.001 for maternal ratings) and depression symptom scores (P=0.008 for teacher ratings) were significant predictors of higher CD symptom scores.

	ODD symptom scores				**CD symptom scores**			
	Maternal rated		Teacher rated		Maternal rated		Teacher rated	
	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P
Pregnancy factors	*N=6,321*		*N=4,431*		*N=6,306*		*N=4,422*	
Maternal smoking	1.11 (0.95-1.30)	0.099	1.15 (0.95-1.40)	0.072	1.33 (1.18-1.51)	**<0.001**	1.13 (0.84-1.53)	0.326
Alcohol use	-	**-**	-	-	1.07 (0.96-1.18)	0.153	1.15 (0.87-1.51)	0.235
Cannabis or hard drug use	1.33 (0.88-2.02)	0.102	1.07 (0.70-1.64)	0.694	1.04 (0.75-1.44)	0.786	1.17 (0.53-2.60)	0.634
Paracetamol use	1.01 (0.89-1.15)	0.798	1.24 (1.05-1.47)	**0.002**	1.10 (0.99-1.22)	0.031	1.26 (0.96-1.65)	0.046
Life events stress score	1.22 (1.07-1.39)	**<0.001**	1.03 (0.86-1.22)	0.718	1.24 (1.11-1.38)	**<0.001**	1.15 (0.87-1.52)	0.235
Anxiety symptom score	1.11 (0.96-1.29)	0.086	1.00 (0.81-1.23)	0.956	1.04 (0.92-1.18)	0.445	0.86 (0.64-1.16)	0.232
Depression symptom score	1.08 (0.93-1.25)	0.234	1.11 (0.89-1.36)	0.282	1.14 (1.01-1.30)	**0.011**	1.23 (0.91-1.66)	0.101

Table 3: Multiple pregnancy factors adjusted for each other in relation to offspring ODD and CD symptom scores. All variables in the models were adjusted for each other as well as for offspring sex, socioeconomic status, young maternal age at delivery, single parent status and comorbid attention-deficit/hyperactivity disorder symptom scores.. CD: conduct disorder. ODD: Oppositional defiant disorder. IRR: incidence rate ratio. P-values indicating significance at α=0.017 (corrected for multiple hypotheses testing) are shown in bold. For all four models, the whole model was significant (P<0.001).

	ODD symptom scores				CD symptom scores			
	Maternal rated		Teacher rated		Maternal rated		Teacher rated	
	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P
Pregnancy factors	*N=6,281*		*N=4,431*		*N=6,270*		*N=4,422*	
Maternal smoking	1.07 (0.92-1.25)	0.287	1.14 (0.95-1.35)	0.087	1.30 (1.15-1.48)	**<0.001**	1.12 (0.87-1.44)	0.281
Alcohol use	-	**-**	-	-	1.05 (0.95-1.17)	0.248	1.08 (:0.87-1.35)	0.381
Cannabis or hard drug use	1.21 (0.80-1.84)	0.264	1.18 (0.73-1.91)	0.402	1.02 (0.76-1.37)	0.889	1.55 (0.69-3.49)	0.197
Paracetamol use	0.98 (0.87-1.12)	0.757	1.22 (1.05-1.42)	**0.002**	1.09 (0.98-1.21)	0.055	1.10 (0.88-1.38)	0.313
Life events stress score	1.18 (1.03-1.35)	**0.002**	1.00 (0.85-1.17)	0.975	1.21 (1.08-1.35)	**<0.001**	1.17 (0.94-1.47)	0.090
Anxiety symptom score	1.11 (0.96-1.28)	0.087	1.06 (0.88-1.28)	0.477	1.04 (0.92-1.18)	0.463	0.91 (0.71-1.17)	0.374
Depression symptom score	1.03 (0.89-1.20)	0.576	1.06 (0.88-1.29)	0.454	1.13 (1.00-1.27)	0.021	1.34 (1.04-1.73)	**0.006**

Table 4: Multiple pregnancy factors adjusted for each other in relation to offspring ODD and CD symptom scores, adjusted for all comorbid disruptive behavior symptomatology. All variables in the models were adjusted for each other as well as for offspring sex, socioeconomic status, young maternal age at delivery, single parent status, comorbid attention-deficit/hyperactivity disorder symptom scores and comorbid ODD or CD symptom scores. CD: conduct disorder. ODD: Oppositional defiant disorder. IRR: incidence rate ratio. P-values indicating significance at α=0.017 (corrected for multiple hypotheses testing) are shown in bold. For all four models, the whole model was significant (P<0.001).

	ODD symptom scores				CD symptom scores			
	Maternal rated		Teacher rated		Maternal rated		Teacher rated	
	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P	IRR (98.3% CI)	P
Pregnancy factors	*N=4,652*		*N=2,898*		*N=4,645*		*N=2,894*	
Maternal smoking	1.07 (0.89-1.29)	0.413	1.24 (0.97-1.58)	0.038	1.30 (1.12-1.51)	**<0.001**	1.20 (0.87-1.67)	0.173
Alcohol use	-	**-**	-	-	1.02 (0.90-1.15)	0.689	1.17 (0.88-1.57)	0.188
Cannabis or hard drug use	1.14 (0.67-1.94)	0.555	1.26 (0.70-2.25)	0.348	0.95 (0.66-1.35)	0.718	1.48 (0.49-4.34)	0.398
Paracetamol use	1.00 (0.86-1.15)	0.973	1.22 (1.00-1.49)	**0.015**	1.09 (0.96-1.23)	0.096	1.09 (0.80-1.49)	0.503
Life events stress score	1.20 (1.03-1.39)	**0.004**	1.00 (0.82-1.22)	0.986	1.25 (1.11-1.42)	**<0.001**	1.04 (0.77-1.40)	0.754
Anxiety symptom score	1.04 (0.89-1.23)	0.590	1.02 (0.79-1.31)	0.871	1.01 (0.88-1.17)	0.813	0.94 (0.66-1.33)	0.659
Depression symptom score	1.02 (0.88-1.23)	0.742	1.02 (0.79-1.32)	0.861	1.09 (0.95-1.25)	0.122	1.44 (1.04-2.00)	**0.008**

Table 5: Multiple pregnancy factors adjusted for each other in relation to offspring ODD and CD symptom scores, adjusted for all comorbid disruptive behavior symptomatology and genetic risk factors. All variables in the models were adjusted for each other as well as for offspring sex, socioeconomic status, young maternal age at delivery, single parent status, comorbid attention-deficit/hyperactivity disorder symptom scores, comorbid ODD or CD symptom scores and genetic risk scores. CD: conduct disorder. ODD: Oppositional defiant disorder. IRR: incidence rate ratio. P-values indicating significance at α=0.017 (corrected for multiple hypotheses testing) are shown in bold. For all four models, the whole model was significant (P<0.001).

Sensitivity analyses concerning paracetamol use and smoking during pregnancy

See **Table S3 (supplementary material)** for all results. We found that after adjustment for common maternal infections and use of other potentially related medication use (in addition to comorbid ADHD, respective ODD or CD symptom scores, and genetic risks scores), paracetamol use during pregnancy remained significantly linked with higher offspring ODD symptoms (P=0.009 for teacher ratings).

See **Table S4 (supplementary material)** for all results. As expected, maternal smoking was related to offspring low birth weight (P<0.001). After further adjusting our model of smoking during pregnancy for low birth weight, maternal smoking during pregnancy remained significantly linked with higher offspring CD symptoms (P<0.001 for maternal ratings).

Discussion

In the present study we investigated a broad range of pregnancy risk factors in relation to both offspring ODD and CD symptomatology in the ALSPAC population cohort. We considered both maternal and teacher ratings of child behavior and adjusted for confounding by comorbid disruptive behavior symptoms and genetic risk scores. Our study adds evidence that the prenatal environmental factors maternal smoking, paracetamol use, life events stress, and depression symptoms during pregnancy are independently associated with offspring ODD and/or CD symptoms in the general population.

Maternal smoking during pregnancy was linked with higher offspring CD symptom scores, whereas we did not find a link with offspring ODD symptoms. Associations between pregnancy smoking and a CD diagnosis have mostly been reported in smaller clinical samples (52,53), while in larger general population samples, such as ALSPAC, maternal smoking during pregnancy has mostly been linked to behavioral questionnaire ratings (8,54,55), which often screen for both ODD and CD (56). The fact that we found specific effects only for CD and not ODD symptoms emphasizes the importance to consider these behavioral disorder dimensions separately and to also adjust for comorbid disruptive symptomatology including ADHD. Tobacco smoke consists of a mixture of chemicals including nicotine, carbon monoxide, polycyclic aromatic hydrocarbons, and heavy metals and causes hypoxia, nutritional deficiencies, and DNA-alterations (57–59). Nevertheless, while smoking during pregnancy has been linked to somatic complications such as low birth weight, birth defects, and adverse development of the nervous system (47,58,60), it has also been suggested that the genetic make-up of the mother might predispose for both harmful behavior during pregnancy and disruptive behavior in offspring (22,23). We found that after adjusting for low birth weight and genetic risk for CD, smoking during pregnancy was still strongly associated with offspring CD symptom scores. However, GWAS-derived genetic risk scores capture only a limited amount of heritability, given effects of rare alleles, gene-environment interactions or epigenetic effects (61,62), thus additional confounds are still likely to play a role. To more rigorously separate inherited and prenatal environmental factors, specific study designs such as in vitro fertilization or surrogacy pregnancy are needed (23,63). In addition, unmeasured factors originating from a shared family environment might also confound reported associations, as a number of sibling-matched studies did not find effects of pregnancy smoking when comparing differentially exposed siblings (22,64,65). However, it is still worth emphasizing that we found an effect of smoking during pregnancy even when adjusting for other pregnancy factors that may be related to smoking and also affect child behavior such as other maternal substance use, life events stressors and internalizing problems (20,21). Furthermore, passive smoking (e.g. by a smoking partner) might also have contributed to the risk of disruptive behavior (66). Therefore, to summarize, we confirm previously reported associations of smoking during pregnancy with offspring CD symptoms in a general population sample, however, we emphasize that causality is not necessarily inferred as more factors are likely to confound the association.

We observed no link between alcohol use during pregnancy and offspring ODD or CD symptomatology. While previous studies reported associations of alcohol use during pregnancy with offspring behavioral problems (10,67,68), most of these studies did not correct for other pregnancy factors such as smoking or mental health issues (e.g., stress, depression) during pregnancy, and also did not take into account comorbid disruptive behavior. Moreover, overlap in genetic risk between CD and alcohol dependence has been previously described (40,69,70), indicating another source of potential confounding. Thus, although alcohol is a notorious harmful substance during pregnancy, we do not report a link with ODD or CD symptom scores in the general population.

In the current study we demonstrated that paracetamol use during pregnancy was specifically associated with offspring ODD symptomatology, while effects specifically on CD symptoms only approached significance. These associations remained significant even after controlling for factors related to paracetamol use such as common maternal infections, accompanying fever, and related medication use (71–73), confirming the results of another ALSPAC-study which also addressed numerous potential confounding factors by adjusting for paracetamol use after birth or by mother's partner, and polygenic risk scores for ADHD (12). However, that study used a screening questionnaire outcome, which does not allow for specific investigation of ODD or CD symptoms, also there was no control for comorbid disruptive behaviors (e.g. ADHD symptoms), and adjustment for other pregnancy factors was limited to smoking and alcohol use only. In addition to offspring oppositional or antisocial behavior, some studies have implicated pregnancy paracetamol use as a risk factor for offspring ADHD (12,74,75). In addition to fever, paracetamol is often used for its analgesic properties. While pain can be a symptom of infection (e.g. as occurring in common rhinovirus or influenza infection), it can also be a symptom associated with chronic stress or due to a number of other somatic causes, which might be related to pregnancy and/or labor. Although we observed clear effects of paracetamol when controlling for maternal stress, it is thus not clear whether the use of paracetamol might also be a proxy for a more complicated course of pregnancy. Considering potential teratogenic mechanisms, animal studies suggested that pharmacological effects on the central nervous system such as interference with brain-derived neurotrophic factor, multiple neurotransmitter systems, or inhibition of the cyclo-oxygenase 2 enzymes might play a role (75). Thus, to conclude, paracetamol use during pregnancy was specifically linked to offspring ODD symptomatology after controlling for a number of confounding variables, confirming related findings of recent literature. Further research should be conducted as underlying biological mechanisms are still largely unknown.

Our study supports an independent association between life events stress during pregnancy and offspring ODD and CD symptomatology. Furthermore, maternal depression scores were linked to higher offspring CD symptom scores. Previous studies reported similar associations of maternal depression and anxiety with offspring behavioral-emotional problems (14,15,37). However, we found that maternal anxiety was only linked to ODD or CD symptoms when considered individually, but not when adjusted for other pregnancy factors, which suggests that this effect might be driven by coexistent stress, depression or other pregnancy factors (20). Regarding biological mechanisms, it has been suggested that

stress-evoked neuroendocrine reactions may affect fetal exposure to glucocorticoids and affect the development of multiple brain structures including parts of the limbic system and prefrontal cortex (76–79). Therefore, future studies should perhaps focus on the role of life events and depression during pregnancy rather than anxiety as risk factors for offspring disruptive behavior symptoms.

Strengths and limitations

Of particular strength has been the use of diagnostic assessments of disruptive behavior symptom dimensions and a broad range of prospectively collected pregnancy measures in a well-powered population sample. This enabled us to address the independent contribution of a variety of pregnancy factors and more robustly investigate relationships with ODD and CD symptomatology. Another strength has been the use of both maternal and teacher ratings. We indeed observed some differences between our analyses of maternal and teacher rated symptom scores, which points to the importance of considering multiple raters as complementary sources of information. Inter-rater discrepancies might reflect a different perspective of child behavior, behavioral variability across different situations, yet also be indications of rating bias (e.g. related to family functioning or psychopathology of the rater) or other sources of measurement error (80,81).

Nevertheless, some limitations need to be acknowledged. First, as mentioned, additional confounding by genetic and familial factors remains an important issue. That is, many assessed pregnancy factors such as life events stress, depression and smoking are likely to persist after birth. Subsequently, chronic stressors and depression may adversely affect parenting quality and family functioning (82), which might promote the development of disruptive behavior (83–85). In addition, the tendency to smoke during pregnancy and/or after birth might also be related to adverse personality traits and psychopathology (82,86). Moreover, both passive smoking and exposure to smoking after birth may also affect children's neurodevelopment and in addition affect breast milk production, composition, and the infant's response to breastfeeding (87,88). Thus future studies should, in addition to genetically informative methods, also take into account postnatal risk factors, such as maternal mood problems or maltreatment, as an additional source of confounding. Second, findings from a population cohort covering a broad range of symptom scores may not necessarily generalize to clinical populations of children diagnosed with ODD or CD. However, risk factors may not fundamentally differ from clinical cases, as these might be considered the extreme ends of the continuous traits distributed within the general population (1,89,90). In addition, the milder levels of ODD and CD symptoms within the general population may explain the relatively small effect sizes in our current study, in particular when considering that ODD and CD are multifactorial conditions. Third, the dichotomization of substance use may have also led to underestimation of effects, given the generally lower prevalence of more severe levels of exposures. A further point of consideration is that associations found in the present study may be specifically related to CD problems during childhood, as different correlates have been suggested for childhood and adolescence onset CD (91). However, as an early onset-age is also predictive of persistence of problems, a certain degree of similarity in risk factors might also be expected

(1). Finally, subjects that could not be included in the analyses due to missing predictor data, showed on average higher symptom scores and exposure rates for certain pregnancy factors than included subjects, which could also indicate an underestimation of effects in our current study.

Conclusions

We conclude that frequently occurring, potentially preventable pregnancy risk factors are specifically linked with offspring ODD and CD symptomatology in the general population. We addressed potential confounding by other pregnancy adversities, comorbid disruptive behavior and genetic factors. While further confounding factors, both genetic and environmental of origin, still pose a challenge for future research, our study adds evidence that independently links pregnancy smoking, paracetamol use, life events stress, and depressive symptoms to specific childhood disruptive behavior symptomatology.

References

1. Olsson M. DSM diagnosis of conduct disorder (CD)--a review. Nord J Psychiatry. 2009;63(Cd):102–12.
2. Boylan K, Vaillancourt T, Boyle M, Szatmari P. Comorbidity of internalizing disorders in children with oppositional defiant disorder. Eur Child Adolesc Psychiatry. 2007;16(8):484–94.
3. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC: American Psychiatric Association; 2013.
4. Waltes R, Chiocchetti AG, Freitag CM. The neurobiological basis of human aggression: A review on genetic and epigenetic mechanisms. Am J Med Genet Part B Neuropsychiatr Genet. 2016;171(5):650–75.
5. Porsch RM, Middeldorp CM, Cherny SS, Krapohl E, van Beijsterveldt CEM, Loukola A, et al. Longitudinal heritability of childhood aggression. Am J Med Genet Part B Neuropsychiatr Genet. 2016;(January):697–707.
6. Latimer K, Wilson P, Kemp J, Thompson L, Sim F, Gillberg C, et al. Disruptive behaviour disorders: a systematic review of environmental antenatal and early years risk factors. Child Care Health Dev. 2012 Sep;38(5):611–28.
7. Maughan B, Taylor A, Caspi A, Moffitt TE. Prenatal Smoking and Early Childhood Conduct Problems: Testing Genetic and Environmental Explanations of the Association. Arch Gen Psychiatry. 2004;61(8):836–43.
8. Murray J, Maughan B, Menezes AMB, Hickman M, Macleod J, Matijasevich A, et al. Perinatal and sociodemographic factors at birth predicting conduct problems and violence to age 18 years: comparison of Brazilian and British birth cohorts. J Child Psychol Psychiatry Allied Discip. 2015;56(8):914–22.
9. Barker ED, Oliver BR, Viding E, Salekin RT, Maughan B. The impact of prenatal maternal risk, fearless temperament and early parenting on adolescent callous-unemotional traits: A 14-year longitudinal investigation. J Child Psychol Psychiatry. 2011;52(8):878–88.
10. Larkby CA, Goldschmidt L, Hanusa BH, Day NL. Prenatal alcohol exposure is associated with conduct disorder in adolescence: Findings from a birth cohort. J Am Acad Child Adolesc Psychiatry. 2011;50(3):262–71.
11. Niclasen J, Nybo Andersen a M, Teasdale TW, Strandberg-Larsen K. Prenatal exposure to alcohol, and gender differences on child mental health at age seven years. J Epidemiol Community Health. 2014;68(3):224–32.
12. Stergiakouli E, Thapar A, Davey Smith G. Association of Acetaminophen Use During Pregnancy With Behavioral Problems in Childhood: Evidence Against Confounding. JAMA Pediatr. 2016;170(10):964–70.
13. Hoover R, Hayes V, Erramouspe J. Association Between Prenatal Acetaminophen Exposure and Future Risk of Attention Deficit/Hyperactivity Disorder in Children. Ann Pharmacother. 2015;49(12):1357–61.
14. Pina-Camacho L, Jensen SK, Gaysina D, Barker ED. Maternal depression symptoms, unhealthy diet and child emotional–behavioural dysregulation. Psychol Med. 2015;45(09):1851–60.
15. Loomans EM, Van der Stelt O, van Eijsden M, Gemke RJBJ, Vrijkotte T, Van den Bergh BR. Antenatal maternal anxiety is associated with problem behaviour at age five. Early Hum Dev. 2011;87(8):565–70.
16. Serati M, Barkin J, Orsenigo G, Altamura A, Buoli M. Research Review: The role of obstetric and neonatal complications in childhood attention deficit and hyperactivity disorder - a systematic review. J Child Psychol Psychiatry Allied Discip. 2017;58(12):1290–300.
17. Luteijn JM, Brown MJ, Dolk H. Influenza and congenital anomalies: A systematic review and meta-analysis. Hum Reprod. 2014;29(4):809–23.
18. Ophelders DRMG, Gussenhoven R, Lammens M, Küsters B, Kemp MW, Newnham JP, et al. Neuroinflammation and structural injury of the fetal ovine brain following intra-amniotic Candida albicans exposure. J Neuroinflammation. 2016;13(1):29.
19. Rotem-Kohavi N, Oberlander TF. Variations in Neurodevelopmental Outcomes in Children with Prenatal SSRI Antidepressant Exposure. Birth Defects Res. 2017;109(12):909–23.
20. Westerneng M, Witteveen AB, Warmelink JC, Spelten E, Honig A, de Cock P. Pregnancy-specific anxiety and its association with background characteristics and health-related behaviors in a low-risk population. Compr Psychiatry. 2017;75:6–13.
21. Little B, Snell L, Gilstrap L, Johnston W. Patterns of multiple substance abuse during pregnancy: implications for mother and fetus. South Med J. 1990;5:507–18.
22. D'Onofrio BM, Van Hulle CA, Waldman ID, Rodgers JL, Harden KP, Rathouz PJ, et al. Smoking during pregnancy and offspring externalizing problems: An exploration of genetic and environmental confounds. Dev Psychopathol. 2008;20(1):139–64.
23. Rice F, Harold GT, Boivin J, Hay DF, Van den Bree M, Thapar A. Disentangling prenatal and inherited influences in humans with an experimental design. PNAS Proc Natl Acad Sci United States Am. 2009;106(7):2464–7.
24. Goodman R. Psychometric properties of the Strengths and Difficulties Questionnaire. J Am Acad Child Adolesc Psychiatry. 2001;40:1337–45.
25. Boyd A, Golding J, Macleod J, Lawlor D, Fraser A, Henderson J, et al. Cohort Profile: the 'children of the 90s' – the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol. 2013;42:111–27.

26. Fraser A, Macdonald-wallis C, Tilling K, Boyd A, Golding J, Davey smith G, et al. Cohort profile: The avon longitudinal study of parents and children: ALSPAC mothers cohort. Int J Epidemiol. 2013;42(1):97–110.
27. Golding J, Pembrey M, Jones R, ALSPAC study team. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol. 2001;15(1):74–87.
28. Goldschmidt L, Day NL, Richardson GA. Effects of prenatal marijuana exposure on child behavior problems at age 10. Neurotoxicol Teratol. 2000;22(3):325–36.
29. Minnes S, Singer LT, Kirchner HL, Short E, Lewis B, Satayathum S, et al. The effects of prenatal cocaine exposure on problem behavior in children 4–10 years. Neurotoxicol Teratol. 2010 Jul;32(4):443–51.
30. Petersen TG, Liew Z, Andersen A-MN, Andersen GL, Andersen PK, Martinussen T, et al. Use of paracetamol, ibuprofen or aspirin in pregnancy and risk of cerebral palsy in the child. Int J Epidemiol. 2017;(December):1–10.
31. Joschko MA, Dreosti IE, Tulsi RS. The teratogenic effects of salicylic acid on the developing nervous system in rats in vitro. Teratology. 1993;48(2):105–14.
32. Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-Amram H, et al. Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. Nat Commun. 2017;8.
33. Haas M, Qu Z, Kim TH, Vargas E, Campbell K, Petrou S, et al. Perturbations in cortical development and neuronal network excitability arising from prenatal exposure to benzodiazepines in mice. Eur J Neurosci. 2013;37(10):1584–93.
34. El Marroun H, White T, Verhulst FC, Tiemeier H. Maternal use of antidepressant or anxiolytic medication during pregnancy and childhood neurodevelopmental outcomes: a systematic review. Eur Child Adolesc Psychiatry. 2014;23(10):973–92.
35. Silva D, Colvin L, Hagemann E, Bower C. Environmental Risk Factors by Gender Associated With Attention-Deficit/Hyperactivity Disorder. Pediatrics. 2014;133(1):e14–22.
36. Huizink AC, Dick DM, Sihvola E, Pulkkinen L, Rose RJ, Kaprio J. Chernobyl exposure as stressor during pregnancy and behaviour in adolescent offspring. Acta Psychiatr Scand. 2007 Dec;116(6):438–46.
37. O'Connor T, Heron J, Golding J, Beveridge M, Glover V. Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. Br J Psychiatry J Ment Sci. 2002;180:502–8.
38. Goodman R, Ford T, Richards H, Gatward R, Multzer H. The Development and Well-Being Assessment: Description and initial validation of an integrated assessment of child and adolescent psychopathology. J Child Psychol Psychiatry Allied Discip. 2000;41(5):645–55.
39. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. IV. American Psychiatric Association; 1994.
40. Dick DM, Aliev F, Krueger RF, Edwards A, Agrawal A, Lynskey M, et al. Genome-wide association study of conduct disorder symptomatology. Mol Psychiatry. 2011;16:800–8.
41. Che R, Motsinger-reif AA. Evaluation of genetic risk score models in the presence of interaction and linkage disequilibrium. Front Genet. 2013;4:1–10.
42. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb). 2005;95:221–7.
43. Karazsia BT, van Dulmen MHM. Regression models for count data: illustrations using longitudinal predictors of childhood injury. J Pediatr Psychol. 2008;33(10):1076–84.
44. Zuur A, Ieno E, Walker N, Saveliev A, Smith G. Zero-Truncated and Zero-Inflated Models for Count Data. In: Mixed effects models and extensions in ecology with R. Springer New York; 2009. p. 261–93.
45. StataCorp. Stata Statistical Software. College Station, TX: Stata Corp LP; 2015.
46. U.K. Office of National Statistics: SOC2000.
47. Pereira P, Da Mata F, Figueiredo A, de Andrade K, Pereira M. Maternal Active Smoking During Pregnancy and Low Birth Weight in the Americas: A Systematic Review and Meta-analysis. Nicotine Tob Res. 2017;19(5):497–505.
48. Knopik V, Marceau K, Palmer R, Smith T, Heath A. Maternal Smoking During Pregnancy and Offspring Birth Weight: A Genetically-Informed Approach Comparing Multiple Raters. Behav Genet. 2010;46(3):353–64.
49. Birtchnell J, Evans C, Kennard J. The total score of the Crown-Crisp Experiential Index: a useful and valid measure of psychoneurotic pathology. Br J Med Psychol. 1988;61 (Pt 3):255–66.
50. Cox J, Holden J, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150:782–6.
51. Murray L, Carothers A. The validation of the Edinburgh Post-natal Depression Scale on a community sample. Br J Psychiatry. 1990;157:288–90.
52. Wakschlag LS, Lahey BB, Loeber R, Green SM, Gordon RA, Leventhal BL. Maternal smoking during pregnancy and the risk of conduct disorder in boys. Arch Gen Psychiatry. 1997;54(7):670–6.
53. Biederman J, Monuteaux MC, Faraone S V, Mick E. Parsing the associations between prenatal exposure to nicotine and offspring psychopathology in a nonreferred sample. J Adolesc Heal. 2009;45(2):142–8.
54. Hutchinson J, Pickett KE, Green J, Wakschlag LS. Smoking in pregnancy and disruptive behaviour in 3-year-old boys and girls: An analysis of the UK millennium cohort study. J Epidemiol Community Health. 2010;64(1):82–8.
55. Brion M-J, Victora C, Matijasevich A, Horta B, Anselmi L, Steer C, et al. Maternal smoking and child psychological problems: Disentangling causal and noncausal effects. Pediatrics. 2010;126(1):e57–65.
56. Goodman RPD. Psychometric Properties of the Strengths and Difficulties Questionnaire. J Am Acad Child Adolesc Psychiatry. 2001;40(11):1337–45.
57. Chernoff N. Teratogenic effects of cadmium in rats. Teratology. 1973;8(1):29–32.
58. Ziaei S, Nouri K, Kazemnejad A. Effects of carbon monoxide air pollution in pregnancy on neonatal nucleated red blood cells. Paediatr Perinat Epidemiol. 2005;19(1):27–30.
59. Mochizuki M, Maruo T, Masuko K, Ohtsu T. Effects of smoking on fetoplacental-maternal system during pregnancy. Am J Obstet Gynecol. 1984;149(4):413–20.
60. Lammer EJ, Shaw GM, Iovannisci DM, Van Waes J, Finell RH. Maternal smoking and the risk of orofacial clefts: susceptibility with NAT1 and NAT2 polymorphisms. Epidemiology. 2004;15(2):150–6.
61. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747–53.
62. Gibson G. Rare and common variants: twenty arguments. Nat Rev Genet. 2012;13(2):135–45.
63. Thapar A, Harold G, Rice F, Ge X, Boivin J, Hay D, et al. Do intrauterine or genetic influences explain the foetal origins of chronic disease? A novel experimental method for disentangling effects. BMC Med Res Methodol. 2007;7:25.
64. Gilman SE, Gardener H, Buka SL. Maternal smoking during pregnancy and children's cognitive and physical development: a causal risk factor? Am J Epidemiol. 2008 Sep 1;168(5):522–31.
65. Obel C, Zhu JL, Olsen J, Breining S, Li J, Grønborg TK, et al. The risk of attention deficit hyperactivity disorder in children exposed to maternal smoking during pregnancy - A re-examination using a sibling design. J Child Psychol Psychiatry Allied Discip. 2016;57(4):532–7.
66. Gatzke-Kopp L, Beauchaine TP. Direct and passive prenatal nicotine exposure and the development of externalizing psychopathology. Child Psychiatry Hum Dev. 2007;38(4):255–69.

67. Hill SY, Lowers L, Locke-Wellman J, Shen S. Maternal smoking and drinking during pregnancy and the risk for child and adolescent psychiatric disorders. J Stud Alcohol. 2000;61(5):661–8.
68. Alvik A, Aalen OO, Lindemann R. Early fetal binge alcohol exposure predicts high behavioral symptom scores in 5.5-year-old children. Alcohol Clin Exp Res. 2013;37(11):1954–62.
69. Kendler KS, Prescott CA, Myers J, Neale MC. The Structure of Genetic and Environmental Risk Factors for Common Psychiatric and Substance Use Disorders in Men and Women. Arch Gen Psychiatry. 2003;60(9):929.
70. Slutske WS, Heath AC, Dinwiddie SH, Madden PA, Bucholz KK, Dunne MP, et al. Common genetic risk factors for conduct disorder and alcohol dependence. J Abnorm Psychol. 1998;107(3):363–74.
71. Dreier JW, Andersen AN, Hvolby A, Garne E, Andersen PK, Berg-beckhoff G. Fever and infections in pregnancy and risk of attention deficit / hyperactivity disorder in the offspring. J Child Psychol Psychiatry. 2016;57(4):540–8.
72. Hornig M, Lipkin WI. Infectious and immune factors in the pathogenesis of neurodevelopmental disorders: epidemiology, hypotheses, and animal models. Ment Retard Dev Disabil Res Rev. 2001;7:200–10.
73. Edwards MJ. Review: Hyperthermia and fever during pregnancy. Birth Defects Res Part A - Clin Mol Teratol. 2006;76(7):507–16.
74. Liew Z, Ritz B, Rebordosa C, Lee P, Olsen J. Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. JAMA Pediatr. 2014;168(4):313–20.
75. de Fays L, Van Malderen K, De Smet K, Sawchik J, Verlinden V, Hamdani J, et al. Use of paracetamol during pregnancy and child neurological development. Dev Med Child Neurol. 2015;57(8):718–24.
76. Kim DR, Bale TL, Epperson CN. Prenatal Programming of Mental Illness: Current Understanding of Relationship and Mechanisms. Curr Psychiatry Rep. 2015;17(2):5.
77. Talge N, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J Child Psychol Psychiatry Allied Discip. 2007;48(3–4):245–61.
78. Van den Bergh B, Mulder E, Mennes M, Glover V. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. Neurosci Behav Rev. 2005;29(2):237–58.
79. Grigoryan G, Segal M. Lasting Differential Effects on Plasticity Induced by Prenatal Stress in Dorsal and Ventral Hippocampus. Neural Plast. 2016;2016:2540462.
80. Martel M, Schimmack U, Nikolas M, Nigg J. Integration of symptom ratings from multiple informants in ADHD diagnosis: a psychometric model with clinical utility. Psychol Assess. 2015;27(3):1060–71.
81. De Los Reyes A. Strategic objectives for improving understanding of informant discrepancies in developmental psychopathology research. Dev Psychopathol. 2013;25(3):669–82.
82. Galbally M, Lewis A. Depression and parenting: the need for improved intervention models. Curr Opin Psychol. 2017;15:61–5.
83. Manly J, Kim J, Rogosch F, Cicchetti D. Dimensions of child maltreatment and children's adjustment: contributions of developmental timing and subtype. Dev Psychopathol. 2001;13(4):759–82.
84. Stouthamer-Loeber M, Loeber R, Homish D, Wei E. Maltreatment of boys and the development of disruptive and delinquent behavior. Dev Psychopathol. 2001;13(4):941–55.
85. Afifi TO, McMillan KA, Asmundson GJG, Pietrzak RH, Sareen J. An examination of the relation between conduct disorder, childhood and adulthood traumatic events, and posttraumatic stress disorder in a nationally representative sample. J Psychiatr Res. 2011;45(12):1564–72.
86. Petfield L, Startup H, Droscher H, Cartwright-Hatton S. Parenting in mothers with borderline personality disorder and impact on child outcomes. Evid Based Ment Health. 2015;18(3):67–75.
87. Napierala M, Mazela J, Merritt TA, Florek E. Tobacco smoking and breastfeeding : Effect on the lactation process , breast milk composition and infant development . A critical review. Environ Res. 2016;151:321–38.
88. Mohamed N, Lov S, Lim P, Al Mamum A, Jan Mohamed H. Early life secondhand smoke exposure assessed by hair nicotine biomarker may reduce children's neurodevelopment at 2years of age. Sci Total Environ. 2018;610–11:147–53.
89. Martin A, Volkmar FR, editors. Lewis's child and adolescent psychiatry: a comprehensive textbook. 4th ed. Wolters Kluwer: Lippincott Williams & Wilkins; 2007.
90. Boden JM, Fergusson DM, Horwood LJ. Risk factors for conduct disorder and oppositional/defiant disorder: Evidence from a New Zealand birth cohort. J Am Acad Child Adolesc Psychiatry. 2010;49(11):1125–33.
91. Frick PJ. Developmental pathways to conduct disorder. Child Adolesc Psychiatr Clin N Am. 2006;15(2):311–31.

Supplementary material chapter 3

ALSPAC genotype data

Children in the ALSPAC sample were genotyped using the Illumina HumanHap550 beadchip array by 23andme subcontracting the Wellcome Trust Sanger Institute (Cambridge UK) and the Laboratory Corporation of America (Burlington, NC, US). Pre-imputation quality control consisted of subject exclusion based on gender mismatches, minimal or excessive heterozygosity, > 3% individual missingness, and insufficient sample replication (identity by descent [IBD] < 0.8). Population stratification was assessed by multidimensional scaling analysis and compared with Hapmap II European descent (CEU), Han Chinese, Japanese, and Yoruba reference populations and all individuals with non-European ancestry were excluded. Single nucleotide polymorphisms (SNPs) with a minor allele frequency < 1%, call rate < 95%, or Hardy-Weinberg equilibrium deviations (P < 5.00E-07) were excluded. Cryptic relatedness was measured as proportion of IDB > 0.1. Related subjects that passed all other quality control thresholds were retained during subsequent phasing (using ShapeIT v2.r727 software) and imputation. Imputation was carried out for 9,115 subjects using Impute2 v.2.2.2 software (using 1000 genomes phase 1 version 3 reference panel), and after further exclusion due to ID-mismatches and consent withdrawal a total of 8,941 subjects were included in our imputed genotype dataset.

Genetic risk scores

Genetic risk scores (GRS) were based on the results of an independent genome-wide association study (GWAS) for conduct disorder (CD) (1). SNPs showing a P-value < 1.00E-05 for either a CD symptom count or CD case status in the original GWAS were extracted from the ALSPAC children's hard-called imputed genome-wide genotype data with PLINK software release 1.9 (available as a free download at https://www.cog-genomics.org/plink2). SNPs were tested for linkage disequilibrium (LD) in the target population (Avon Longitudinal Study of Parents and Children sample consisting of mainly European individuals) using the web-based application suite LD-link (available at https://analysistools.nci.nih.gov/LDlink/). We considered SNPs to be in LD when they showed r^2>0.6. From (a block of) SNPs in LD, the SNP with the strongest individual signal was included in the GRS. From the 29 total SNPs that were identified with a P-value < 1.00E-05 for CD symptom count or case status in the base study, 7 SNPs were excluded because of LD, and 5 SNPs were excluded because of a minor allele frequency < 0.01, resulting in a total of 17 SNPs that were included in the GRS. The GRS were computed for each individual as a

weighted sum of the SNP and its explained variance, as described by Che et al. (2013) (2). This method takes into account both the original effect size and allele frequency and showed robust results in cases of SNP-interaction, LD and false-positives (2). Rather than the log-transformed odds ratio we used the beta regression coefficient for the CD symptom count GWAS in computing SNP weights, resulting in the following equation:

$$GRS = \sum_{i=1}^{17} \left(\beta_i \sqrt{2MAF_i(1 - MAF_i)} G_i \right)$$

GRS = individual genetic risk score. β = beta regression coefficient for *i*-th SNP from original GWAS on CD symptom count. MAF = minor allele frequency for *i*-th SNP from original GWAS. G = number of risk alleles for *i*-th SNP from original GWAS. Modified equation from explained variance weighted model in Che et al. (2013) (2).

Young maternal age at delivery	Maternal age < 20 at delivery.
Maternal low socioeconomic status	U.K. National Statistics office SOC2000-classification (3) (social class based on occupation) was used as SES-proxy. 'Low SES' was defined as social classes IV ('partially skilled job') or V ('unskilled job'). SES was assessed at 18 weeks gestation.
Maternal single parent status	Mother lives alone or has no partner
Pregnancy variables, assessed at 18 weeks gestation	
Maternal smoking	Any amount of smoking during the first trimester of pregnancy.
Alcohol use	Any amount of alcohol consumption during the first trimester of pregnancy.
Cannabis or hard drugs use	Any amount of cannabis or hard drug use during the first trimester of pregnancy.
Influenza	Any influenza infection during pregnancy.
Candidiasis	Any candidiasis infection during pregnancy.
Urinary tract infection	Any urinary tract infection during pregnancy.
Medication for infection	Any (amount of) medication use for infection, no further pharmacological specification.
Paracetamol use	Any amount of paracetamol use during pregnancy.
Aspirin use	Any amount of aspirin use during pregnancy.
Life event stress score	Weighted stress score over 42 life events stressors using a 5 point scale (0-4) resulting in a total possible rang of 0 to 164 (median-split).
Anxiety symptom score	Crown Crisp Experience Index (CCEI) subscale score of anxiety during pregnancy. Possible range 0 to 16 (median-split) (4).
Depression symptom score	Edinburgh Postnatal Depression Scale (EPDS) of depression symptoms, which has also been validated for prenatal use (5,6).
Medication for anxiety or depression	Any (amount of) medication use for anxiety or depression, no further pharmacological specification.
Low birth weight	Birth weight < 2500 grams.
Preterm birth	Birth before 37 weeks of gestation.

Table S1: Variable definitions not described in the main text.

	Maternal ratings			Teacher ratings		
	Included	Excluded	P	Included	Excluded	P
Offspring ODD symptom score	1.35 ± 2.80 (N=6,321)	1.57 ± 3.04 (N=1,789)	0.005	1.39 ± 3.07 (N=4,431)	2.11 ± 3.94 (N=1,898)	<0.001
Offspring CD symptom score	0.55 ± 1.03 (N=6,306)	0.64 ± 1.14 (N=1,825)	0.002	0.34 ± 1.24 (N=4,422)	0.66 ± 1.84 (N=1,907)	<0.001

Table S2a: Differences between subjects with and without complete pregnancy data. Independent t-tests between subjects with and without complete pregnancy data for statistical analyses involving multiple pregnancy variables.

Because subjects without complete pregnancy data showed on average higher ODD and CD symptom scores than subjects with complete pregnancy data, we investigated also any potential differences between *these* two groups on relevant pregnancy factors (**Table S2b**). Note that this involves only subjects who were rated by mother or teacher at age 7;9 years. We do not highlight any significance because multiple tests were conducted for partially

overlapping samples. A slightly overcorrected threshold would be approximated by 0.05 / 18 (total number of tests) ≈ 0.0027.

	Maternal ratings			Teacher ratings		
	Included	Excluded	P	Included	Excluded	P
Maternal smoking	18.6%	22.3%	0.001	22.1%	27.5%	<0.001
Alcohol use	55.9%	54.5%	0.303	55.4%	53.5%	0.199
Cannabis or hard drug use	2.1%	3.0%	0.063	2.0%	3.6%	0.007
Paracetamol use	53.0%	55.5%	0.064	54.0%	58.4%	0.004
Life events stress scores	48.1%	48.0%	0.943	48.1%	46.9%	0.509
Anxiety symptom scores	44.5%	50.0%	0.001	45.5%	52.3%	<0.001
Depression symptom scores	43.8%	53.3%	<0.001	45.1%	53.8%	<0.001

Table S2b: Differences between subjects with and without complete pregnancy data, pregnancy factors. Independent t-tests between subjects with and without complete pregnancy data for statistical analyses involving multiple pregnancy variables

	Teacher rated ODD symptom scores (*N=2,885*)	
Pregnancy factors	IRR (98.3% CI)	P
Maternal smoking	1.24 (0.97-1.59)	0.034
Cannabis or hard drug use	1.23 (0.69-2.18)	0.385
Paracetamol use	1.25 (1.02-1.53)	**0.009**
Life events stress score	1.00 (0.82-1.22)	0.986
Anxiety symptom score	1.02 (0.80-1.32)	0.827
Depression symptom score	1.02 (0.79-1.32)	0.846
Influenza	0.88 (0.64-1.20)	0.326
Candidiasis	1.05 (0.82-1.36)	0.624
Urinary tract infection	1.20 (0.82-1.75)	0.260
Medication for infection	0.95 (0.72-1.25)	0.659
Aspirin use	0.99 (0.64-1.52)	0.947

Table S3: Sensitivity analyses investigating further potential confounding factors for paracetamol use during pregnancy. All variables in the models were adjusted for each other as well as offspring sex, socioeconomic status, young maternal age (age < 20) at delivery, single parent status, comorbid ADHD symptom scores, comorbid ODD or CD symptom scores and genetic risk scores. IRR = incidence rate ratio. P-values indicating significance at α=0.017 are shown in bold. The whole model was significant (P<0.001).

	Maternal rated CD symptom scores (*N=4,585*)	
Pregnancy factors	IRR (98.3% CI)	P
Maternal smoking	1.32 (1.14-1.53)	**<0.001**
Alcohol use	1.02 (0.90-1.15)	0.699
Cannabis or hard drug use	0.95 (0.66-1.36)	0.731
Paracetamol use	1.09 (0.97-1.23)	0.091
Life events stress score	1.25 (1.11-1.42)	**<0.001**
Anxiety symptom score	1.02 (0.89-1.17)	0.752
Depression symptom score	1.08 (0.94-1.25)	0.164
Low birth weight	0.75 (0.53-1.06)	0.048

Table S4: Sensitivity analyses investigating low birth weight as confounding factor for smoking during pregnancy. All variables in the model were adjusted for each other as well as offspring sex, socioeconomic status, young maternal age (age < 20) at delivery, single parent status, comorbid ADHD symptom scores, comorbid ODD or CD symptom scores and genetic risk scores. IRR = incidence rate ratio. P-values indicating significance at α=0.017 are shown in bold. The whole model was significant (P<0.001). Of note, maternal smoking also predicted a low birth weight case-control status in our sample (OR=1.83 (95% CI 1.54-2.19), P<0.001), similar to existing literature (7,8).

Supplementary material references

1. Dick DM, Aliev F, Krueger RF, Edwards A, Agrawal A, Lynskey M, et al. Genome-wide association study of conduct disorder symptomatology. Mol Psychiatry [Internet]. 2011;16:800–8. Available from: http://dx.doi.org/10.1038/mp.2010.73
2. Che R, Motsinger-reif AA. Evaluation of genetic risk score models in the presence of interaction and linkage disequilibrium. Front Genet. 2013;4:1–10.
3. U.K. Office of National Statistics: SOC2000.
4. Birtchnell J, Evans C, Kennard J. The total score of the Crown-Crisp Experiential Index: a useful and valid measure of psychoneurotic pathology. Br J Med Psychol [Internet]. 1988;61 (Pt 3):255–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3179248
5. Cox J, Holden J, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150:782–6.
6. Murray L, Carothers A. The validation of the Edinburgh Post-natal Depression Scale on a community sample. Br J Psychiatry. 1990;157:288–90.
7. Pereira P, Da Mata F, Figueiredo A, de Andrade K, Pereira M. Maternal Active Smoking During Pregnancy and Low Birth Weight in the Americas: A Systematic Review and Meta-analysis. Nicotine Tob Res. 2017;19(5):497–505.
8. Knopik V, Marceau K, Palmer R, Smith T, Heath A. Maternal Smoking During Pregnancy and Offspring Birth Weight: A Genetically-Informed Approach Comparing Multiple Raters. Behav Genet. 2010;46(3):353–64.